Supporting information

Photo/biocatalytic system for visible-light driven L-alanine production from ammonia and pyruvate

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1. UV-vis absorption spectrum of zinc meso-tetra(4-sulfonatophenyl)porphyrin tetrasodium salt ($ZnTPPS^{4-}$)

Figure S1 shows the UV-vis absorption spectrum of $ZnTPPS^{4-}$ in the 2-[4-(2-hydroxyethyl)-1-piperazinyl]- ethanesulfonic acid (HEPES)-NaOH buffer solution.

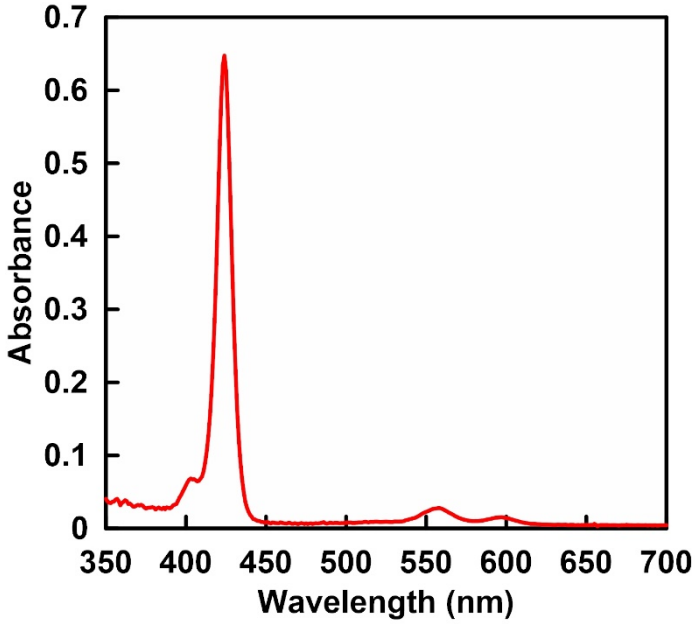

Figure S1. UV-vis absorption spectrum of $ZnTPPS^{4-}$ in HEPES-NaOH buffer solution.

2. Determination for L-alanine concentration using high performance liquid chromatography (HPLC)

The concentration of L-alanine was determined using high performance liquid chromatography system (HPLC; Hitachi High-Tech Corporation Chromaster) with reversed phase column (InertSustainSwift C18 GL Sciences In). The 0.1 % trifluoroacetic acid in aqueous solution and acetonitrile were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 1.0 mL min^{-1}. Fluorescent labelling of L-alanine was carried out by adding L-alanine to a mixture of borate-NaOH buffer and acetonitrile containing 4-fluoro-7-nitro-2,1,3-benzo oxadiazole (NBD-F) and allowing the mixture to stand at 60°C for 5 min and in the freezer for 2 min, followed by addition of a 1.0% HCl solution. The retention time for L-alanine was detected at 21.3-21.8 min. The fluorescence changes (excitation and emission wavelengths were 430 and 530 nm, respectively) in the various L-alanine concentrations (0 – 2.0 mM) during the HPLC analysis were shown in Figure S2. Inset of Figure S1 shows the relationship between the L-alanine concentration and the detection peak area using HPLC.

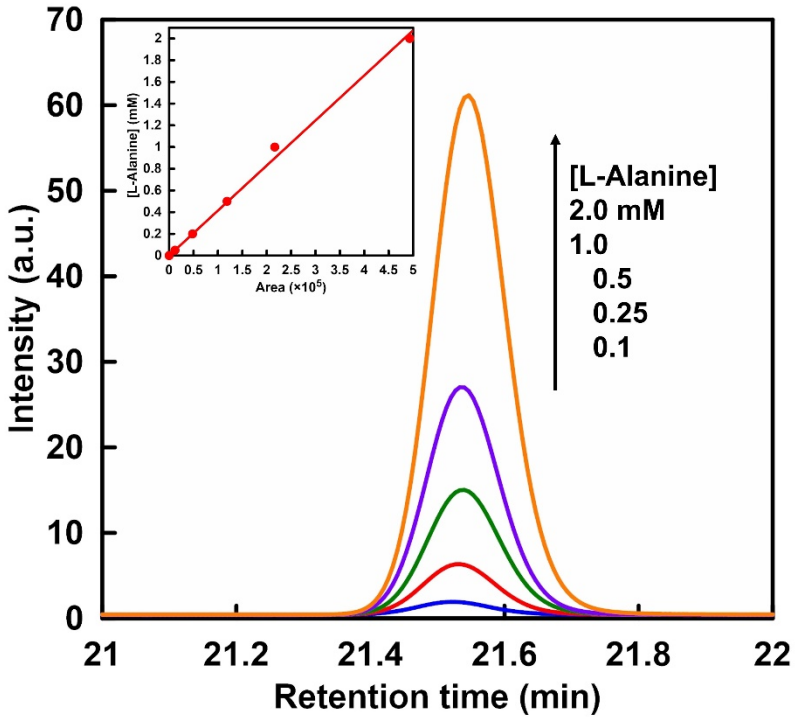

Figure S2. Chromatogram of L-alanine (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 8.0). Inset: Relationship between the detection peak area and the L-alanine concentration.

As shown in the inset of Figure S2, the detected peak area and the L-alanine concentration showed a good linear relationship (correlation coefficient: r^2=0.998) as following equation (S1).

$$[\text{L-alanine}]\ (\text{mM}) = 4.2\times10^{-6}\times\text{Peak area} \quad \text{(S1)}$$

3. A chart of HPLC sampled from the reaction solution of sodium pyruvate, ammonium bicarbonate, NADH, and AlDH in HEPES-NaOH buffer solution

Figure S3 shows HPLC chart of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) during incubation.

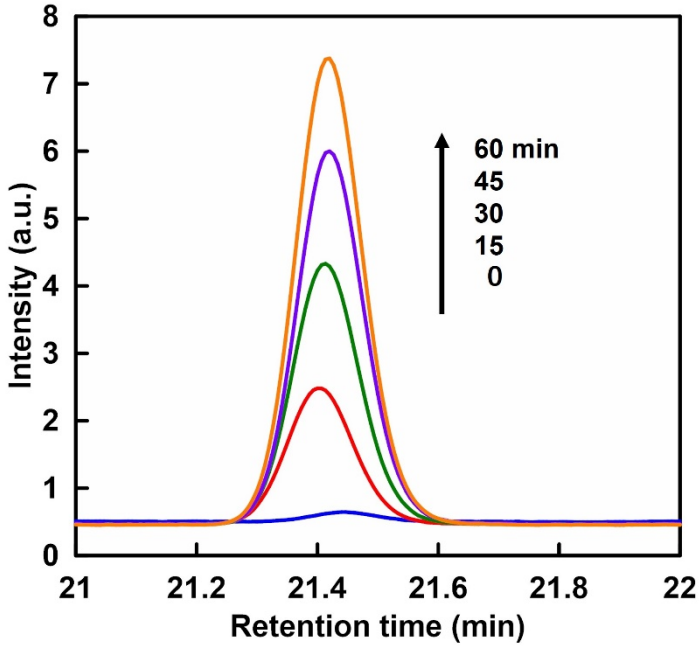

Figure S3. Chromatogram of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) during incubation.

Figure S4 shows HPLC chart of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (0-300 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) after 3 min incubation. Figure S5 shows HPLC chart of a sample consisting of sodium pyruvate (0 – 4.0 mM), ammonium bicarbonate (5.0 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) after 3 min incubation.

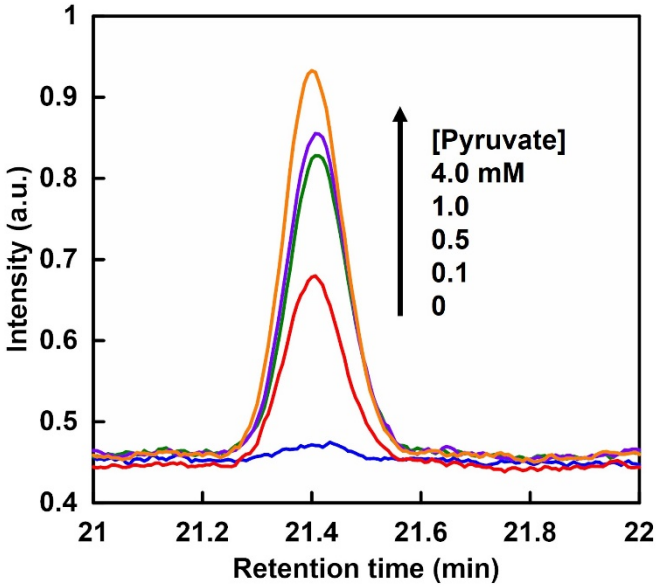

Figure S4. Chromatogram of a sample consisting of sodium pyruvate (0-4.0 mM), ammonium bicarbonate (5.0 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) after 3 min incubation.

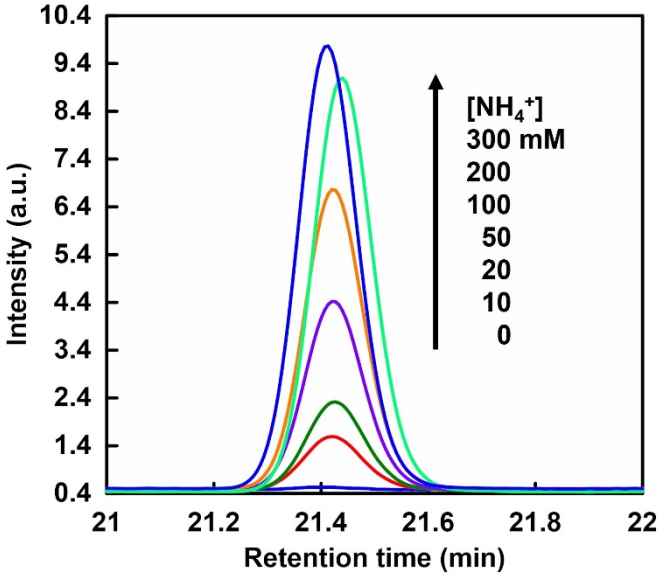

Figure S5. Chromatogram of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (0 - 300 mM), NADH (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer solution (pH 8.0) after 3 min incubation

4. Determination for pyruvate concentration using ion chromatography

The amount of pyruvate was detected using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 x 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for pyruvate was detected at 8.5-9. 0 min. The electrical conductivity changes in the various pyruvate concentrations (0 – 1.0 mM) were shown in Figure S6. Inset of Figure S6 shows the relationship between the detection peak area and the pyruvate concentration using ion chromatograph.

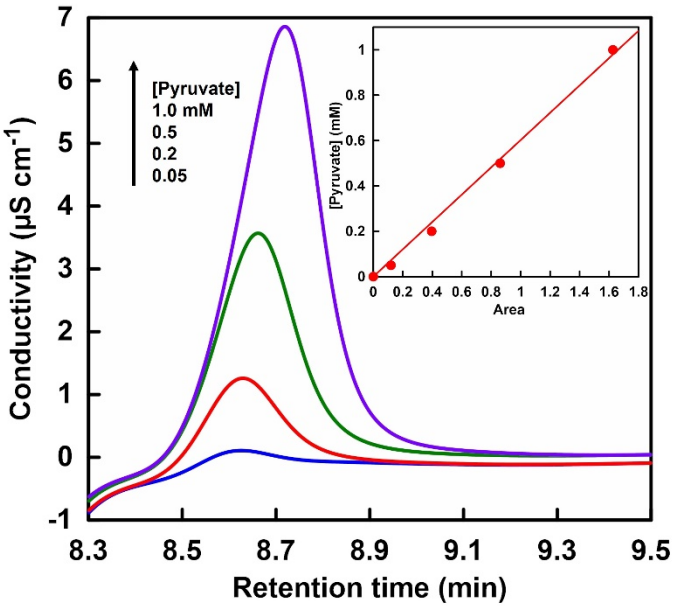

Figure S6. Chromatogram of pyruvate (0 – 1.0 mM) in 500 mM-HEPES buffer (pH 8.0). Inset: Relationship between the detection peak area and the pyruvate concentration.

As shown in the inset of Figure S6, the pyruvate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.998) as following equation (S2).

$$[\text{pyruvate}](\text{mM}) = 0.6 \times \text{Peak area} \qquad \text{(S2)}$$

5. A chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate, ammonium bicarbonate, TEOA, $ZnTPPS^{4-}$, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and AlDH in HEPES-NaOH buffer with visible light irradiation

Figure S7 shows an ion chromatography chart of the sample solution containing sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation.

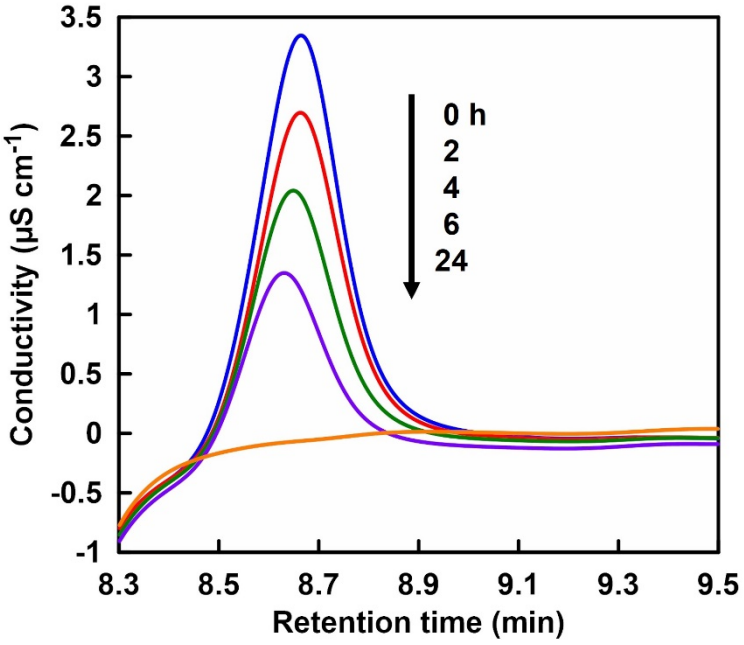

Figure S7. Chromatogram of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation.

6. A chart of HPLC sampled from the reaction solution of sodium pyruvate, ammonium bicarbonate, TEOA, $ZnTPPS^{4}$, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and AlDH in HEPES-NaOH buffer with visible light irradiation

Figure S8 (a) shows an HPLC chart of the sample solution containing sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation.

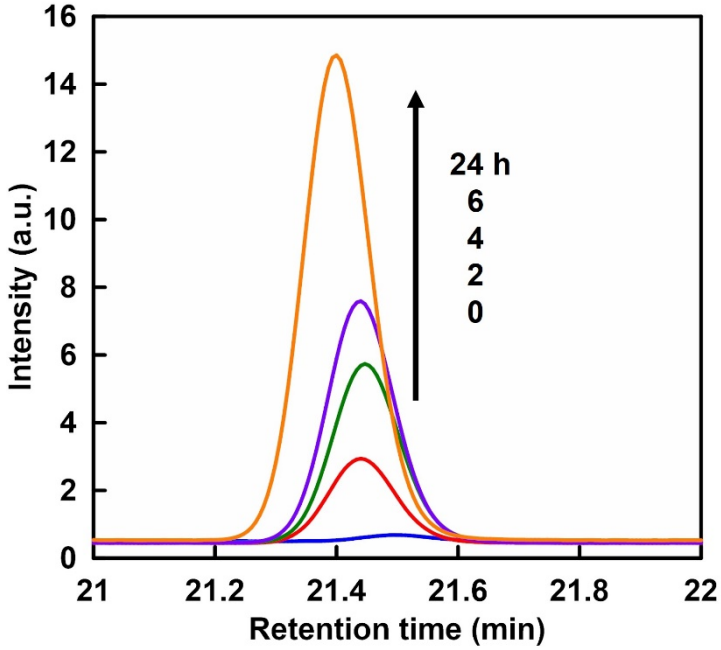

Figure S8(a). Chromatogram of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation.

Figure S8 (b) shows an HPLC chart of the sample solution containing sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (0.1 or 1.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation.

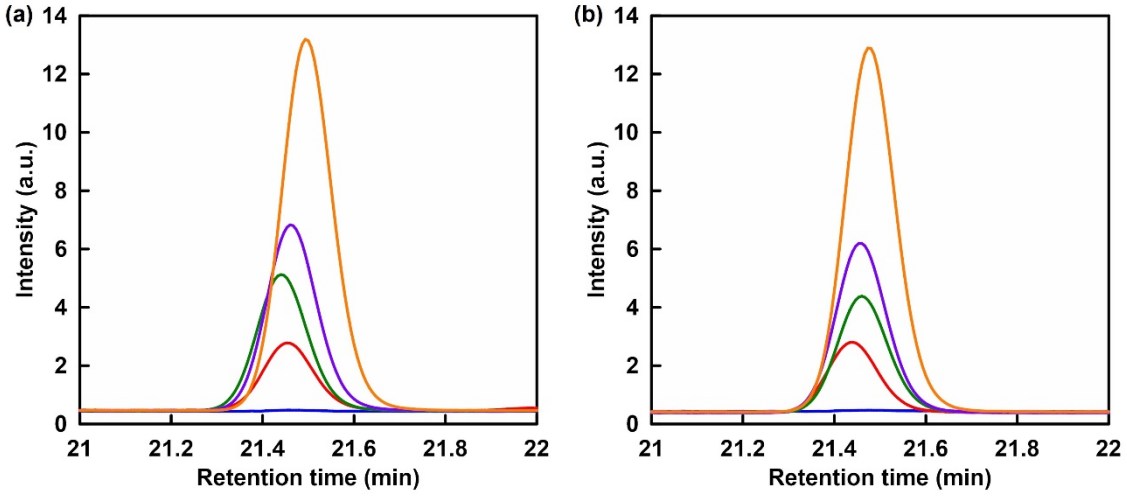

Figure S8(b). Chromatogram of a sample consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) with visible light irradiation. (a) $[NAD^+]$=0.1 mM; (b) 0.25 mM.

7. Measurement for the quantum yield of L-alanine production using the system of sodium pyruvate, ammonium bicarbonate, TEOA, $ZnTPPS^{4-}$, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and AlDH in HEPES-NaOH buffer with monochromatic light irradiation

Figure S9 shows the experimental setup for the measurement of the quantum yield of L-alanine production with the system of sodium pyruvate, ammonium bicarbonate, TEOA, $ZnTPPS^{4-}$, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and AlDH under monochromic light irradiation. The quantum yield measurement system consists of a light source for monochromatic light irradiation (75 W Xe lamp; OB-75X Optical Building Blocks), a temperature-controlled cell holder (QPod 2e/AbsKit; Quantum Northwest Inc.), a light source for monitoring (DH-2000-BAL 25 W D_2 lamp and tungsten-halogen lamp; Ocean Optics), and a spectrophotometer (USB4000-UV-Vis.; Ocean Optics). Monochromatic light at 420, 500, 550 and 635 nm was irradiated to the samples through bandpass filters (bandwidth 10 nm; ThorLabs) for the Xe lamp light sources, respectively. The monochromatic light intensity was measured using an optical power meter (Model 3664; HIOKI E.E. CORPORATION).

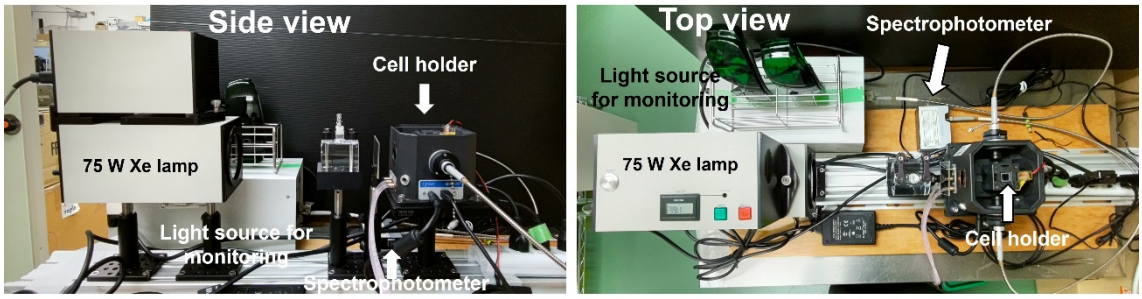

Figure S9. Experimental setup for the measurement of the relative quantum yield of L-alanine production with the system of sodium pyruvate, ammonium bicarbonate, TEOA, $ZnTPPS^{4-}$, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ and AlDH under monochromic light irradiation.

For the L-alanine production monochromic light irradiation, a solution consisting of sodium pyruvate (0.5 mM), ammonium bicarbonate (5.0 mM), TEOA (0.2 M), $ZnTPPS^{4-}$ (10 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (10 μM), NAD^+ (2.0 mM) and AlDH (0.1 U; *ca*. 2.6 μM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.0) was deaerated by freeze-pump-thaw cycles repeated 6 times and then introduced in gas phase with the Ar gas for 10 min. The sample solution was irradiated with monochromic light at room temperature using the experimental setup shown in Figure S9. The relative quantum yield was determined by

dividing the amount of L-alanine produced by monochromatic light irradiation over a period of time by the number of photons absorbed by $ZnTPPS^{4-}$. Figure S10 shows wavelength dependence of the relative quantum yields for L-alanine production after 2 h irradiation.

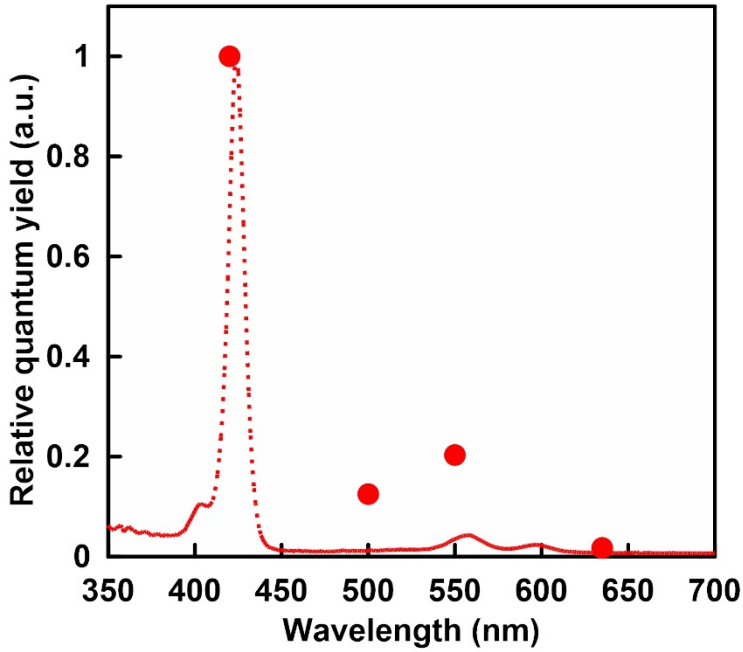

Figure S10. Wavelength dependence of the relative quantum yields for L-alanine production. Dotted line indicates UV-vis absorption spectrum of $ZnTPPS^{4-}$.

8. L-alanine production from pyruvate and ^{15}N-labeled ammonium bicarbonate with the system of TEOA, ZnTPPS^{4-}, [Cp*Rh(bpy)(H$_2$O)]$^{2+}$, NAD$^+$ and AlDH in HEPES-NaOH buffer with irradiation

Figure S11 shows the ^{1}H-NMR (a) and ^{15}N-NMR (b) of the sample solution after 24 h irradiation.

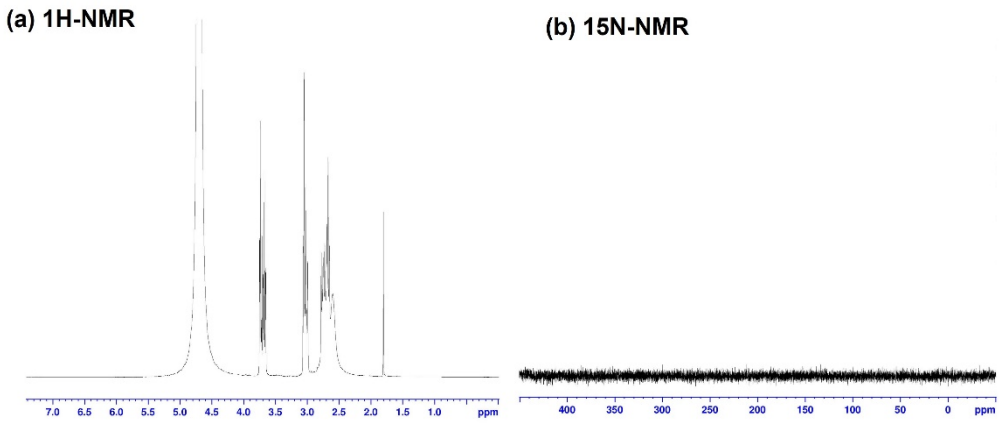

Figure S11. ^{1}H-NMR (a) and ^{15}N-NMR (b) of the sample solution after 24 h irradiation.